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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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MEMORANDUM

DATE: December 20, 2007

SUBJECT: CYHALOFOP-BUTYL: Evaluation of Mode of Action Data and Classification of Carcinogenicity

PC Code: 082583

TO: Alan C. Levy
Registration Action Branch 2
Health Effects Division (7509P)

THROUGH: William Burnam, Chair
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FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
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On October 3, 2007, HED scientists (W. Burnam, V. Dellarco, J. Kidwell, A. Levy, M. Manibusan, E. Mendez, and J. Rowland) met to discuss whether activation of the peroxisome proliferation receptor alpha (PPARα) is the primary mode of action (MOA) in the liver for cyhalofop-butyl. The MOA studies in the mouse were evaluated to determine if these data supported a waiver for repeating (at higher doses) the chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice.

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CYHALOFOP-BUTYL

I. Background

A 104-week rat chronic toxicity/carcinogenicity study [(MRID 45000417); June 2, 1994; HDT (mg/kg/day): M = 3.44, F = 25)] and a 78-week mouse carcinogenicity study [(MRID 45000418); June 2, 1994: HDT (mg/kg/day): M = 10.06, F = 10.28)] were submitted to the Agency to fulfill the guidelines for cancer assessment. There was no evidence of carcinogenicity in either study at the doses tested; however, neither of these studies was considered to reach a maximum tolerated dose. Based on chronic toxicity data generated with this herbicide and carcinogenicity data for other herbicides of the aryloxyphenoxy-propionate chemical family, it was acknowledged that the liver was the primary target organ and peroxisome proliferation was a common effect of other aryloxyphenoxy herbicides. It was, therefore, concluded that cyhalofop-butyl was most likely to operate via a PPARα-mediated hepatocarcinogenic rodent mode of action (MOA).

In an August 13, 2002 meeting with HED and RD (memo dated September 12, 2002), the Registrant agreed to conduct studies to show that cyhalofop-butyl is a peroxisome proliferator and activation of the peroxisome proliferation receptor alpha (PPARα) would be the primary MOA for the induction of liver tumors in mice. The lack of human relevance for liver tumors formed via this mode of action would then obviate the need for repeating the carcinogenicity studies at higher doses.

II. Mode of Action Studies

The Registrant chose to conduct the mode of action studies rather than repeat the long term rat and mouse studies. The MOA studies submitted included the following:

1. 28-Day Evaluation of Peroxisome Proliferation in Mice (MRID 46471101); January 14, 2004.

2. *In Vitro* Activity in a Peroxisome Proliferator Activated Receptor-Alpha Reporter Assay (MRID 46471102); April 5, 2004.

3. Discussion of Mechanistic Data in the Mouse in Support of a Waiver for Repeating Chronic Studies (MRID 46471103); February 3, 2005.

III. Evidence Supporting the PPARα Proposed MOA

The registrant provided mechanistic studies in which cyhalofop-butyl was clearly shown to induce peroxisome proliferation in mouse liver when administered at doses comparable to and above those used in the carcinogenicity studies. Administration of cyhalofop-butyl to mice resulted in a number of responses in the liver which were consistent with the proliferation of hepatocellular peroxisomes and included: increased liver weight, hepatocellular hypertrophy, the induction of acyl-CoA oxidase enzyme activity (very specific enzyme change for this MOA), and peroxisomal volume density. These effects showed both a dose-response and temporal concordance. Hepatocellular necrosis and degeneration occurred at the highest dose level and were attributed to excessive cellular stress beyond the ability of the cells to adapt. All effects induced by cyhalofop-butyl were reversible following a 28-day recovery period. The spectrum of changes noted in this study demonstrate that cyhalofop-butyl is a weak hepatic peroxisome proliferator.

Mechanistic studies submitted were supportive of the postulated PPARα mode of action in the liver. Specifically, it was concluded that adequate and sufficient evidence were provided for the following key causal events:

1. *Activation of PPARα with reporter assay:* The purpose of this study was to assess the ability of the test compound to bind to and activate the peroxisome proliferator activated receptor-alpha (PPAR-α) utilizing an *in vitro* receptor-reporter transactivation assay. Cyhalofop-butyl produced a concentration-dependent induction of luciferase activity at 10 µM and above, with a maximum increase of 3.0-fold over DMSO controls (Table 1). Under the conditions of this study, cyhalofop-butyl binds to the PPAR-α receptor and activates a downstream reporter gene. Cyhalofop-butyl is considered a weak PPAR-α agonist in comparison to the potent agonist, WY14643. Because the *in vitro* reporter assay is highly specific to the PPARα MOA, it adds to the weight of evidence that demonstrates this to be the primary liver tumor mode of action for cyhalofop-butyl.

Table 1. Concentration-response assay (fold-induction relative to DMSO controls) in transfected cells treated with cyhalofop-butyl, WY14643, ciglitazone, or TPA for 24 hours[a]

Treatment	Concentration (molar)									
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	$2x10^{-5}$ M	$5x10^{-5}$ M	10^{-4} M	$2x10^{-4}$ M	$5x10^{-4}$ M
Cyhalofop-butyl	---	---	1.17	1.01	1.26	1.89	2.66	2.81	2.73	2.99
WY14643[b]	1.04	1.52	2.83	6.00	7.15	---	---	7.61	---	---
Ciglitazone[c]	1.21	1.23	1.22	1.73	2.17	---	---	---	---	---
TPA[d]	0.92	0.5	0.33	0.29	0.11*	---	---	---	---	---

a Data were obtained from page 20 of MRID 46471102
b Positive control activator
c PPAR-γ specific activator
d Non-activator
--- Concentration not used
* Indicates cytotoxicity as determined by decreased β-galactosidase activity.

2. *In vivo evidence demonstrating dose response and temporal concordance for precursor events (e.g., histological evidence of increase in the number of subcellular organelles and size of peroxisomes, increased acyl CoA oxidase activity, hepatic cell proliferation).*

In a 28-day peroxisome proliferation study, mice were exposed to dietary dose levels of 0, 0.5, 5, 50 or 150 mg/kg/day for either 7 or 28 days. Peroxisomal acyl-CoA oxidase activity was increased over controls in the males dosed with ≥5 mg/kg/day and in the females dosed with ≥50 mg/kg/day at day 28 (Table 2). These increases were reversible in the 50 mg/kg/day group, as activity returned to control levels by Day 56. The Acyl CoA data was very supportive, robust, and consistent with PPARα mode of action. BrdU labeling index was slightly increased in the 150 mg/kg/day males and females. Peroxisome volume density in the hepatocytes was increased in the ≥0.5 mg/kg/day males and ≥5 mg/kg/day females (Table 3). These findings were also reversible at 50 mg/kg/day after a 28-day recovery period. Overall the cell proliferation data were consistent with a weak mitogenic stimulus (weak agonist). In addition, the presence of hepatocellular hypertrophy (≥5 mg/kg/day), increased liver weights (≥50 mg/kg/day), slight necrosis ((≥50 mg/kg/ay) as well as increased liver enzymes (≥50 mg/kg/day) seen at 7 and 28 days was consistent with a mitogenic response.

TABLE 2. Mean (±SD) peroxisomal acyl-CoA oxidase activity in the liver of mice treated with cyhalofop-butyl for up to 28 days with a 28-day recovery period.[a]

Study Day	Dose (mg/kg/day)				
	0	0.5	5	50	150
Males					
Day 28	9.272±2.188	12.658±2.225	35.837±13.833* (↑287)	126.19±20.869* (↑1261)	179.73±26.843* (↑1838)
Day 56	12.917±3.237	---	---	14.321±3.135	---
Females					
Day 28	6.799±1.831	8.266±5.478	13.176±4.318	57.604±27.507* (↑747)	125.99±19.333* (↑1753)
Day 56	12.379±3.667	---	---	12.075±3.179	---

a Data were obtained from Tables 15 and 16 on pages 76-79 of MRID 46471101. Percent differences from controls, calculated by reviewers, are included in parentheses. n = 10

* Significantly different from controls; p≤0.05

--- Not applicable

TABLE 3. Mean (±SD) peroxisome volume density (%) in the liver of mice treated with cyhalofop-butyl for up to 28 days with a 28-day recovery period.[a]

Study Day	Dose (mg/kg/day)				
	0	0.5	5	50	150
Males					
Day 28	2.09±0.49	3.04±0.80* (↑45)	4.72±1.24* (↑126)	12.79±2.14* (↑512)	18.50±3.32*(↑785)
Day 56	2.61±0.63	---	---	2.79±0.54	---
Females					
Day 28	2.06±0.41	1.92±0.44	2.97±0.66* (↑44)	6.18±1.71* (↑200)	10.88±2.85* (↑428)
Day 56	1.74±0.54	---	---	1.51±0.29	---

a Data were obtained from Tables 19 and 20 on pages 86-89 of MRID 46471101 Percent differences from controls, calculated by reviewers, are included in parentheses. n = 10

* Significantly different from controls; p≤0.05

--- Not applicable

Morphological changes typical of a mitogenic MOA, including increased liver weight and liver hypertrophy, were seen in rat and mouse subchronic and chronic toxicity studies at doses of ≥25 mg/kg/day for rats and ≥10 mg/kg/day for mice (Table 4). These data strengthen the weight of evidence for demonstrating that a PPARα mode of liver carcinogenic action is operative.

Table 4. Repeated-Dose Toxicity of Cyhalofop-Butyl in Rats and Mice

Study	Treatment-Related Histopathological Effects	Reference
Subacute Sprague-Dawley Rat 0, 25, 400 (M), 800 (F), 1600 mg/kg/day	Increased liver weight and hepatocellular hypertrophy at ≥25 (M) and ≥800 (F) mg/kg/day.	Corley, 1991a MRID 45000413
Subacute CD-1 Mice 0, 10, 30, 100, 350 mg/kg/day	Increased liver weight and hepatocellular hypertrophy at ≥10 mg/kg/day (MF); Necrosis at ≥100 mg/kg/day (MF).	Corley, 1991b MRID 45000412
Subacute CD-1 Mice 0, 0.5, 5, 50, 150 mg/kg/day	Increased liver weight and hepatocellular hypertrophy at ≥5 mg/kg/day (MF); Necrosis at ≥50 (M) & 150 (F) mg/kg/day.	Yano and Day, 2004 MRID 46471101
Subchronic Sprague-Dawley Rat 0, 3, 25, 100, 400 (M); 0, 10, 100, 400, 800 (F) mg/kg/day	Increased liver and kidney (M) weights and hepatocellular hypertrophy at ≥25 (MF); Hepatocellular necrosis at ≥100 mg/kg/day (M)	Corley, 1991a MRID 45000413
Subchronic Fischer (344/DuCrj) Rat 0, 30, 300, 1000, 3000 ppm (0, 1.7/2.0, 17/20, 61/65, 190/200 mg/kg/day)	Increased liver weight and hepatocellular hypertrophy at ≥17 (M) and ≥61 (F) mg/kg/day; Kidney effects - increased lipofuscin deposition (MF) and decreased acidophilic bodies (M) at 190/200 mg/kg/day.	Harada, 1993a MRID 45014705
Subchronic CD-1 Mouse 0, 1(M), 3, 10, 30, 100 (F) mg/kg/day	Increased liver weights and hepatocellular hypertrophy at ≥10 (MF); Hepatocellular necrosis at ≥100 mg/kg/day (M).	Corley, 1991b MRID 45000412
Subchronic CD-1 Mouse 0, 3, 30, 100, 300 ppm (0, 0.37/0.44, 3.6/4.3, 12/14, 38/41 mg/kg/day)	Increased liver weight and hepatocellular hypertrophy, inflammation and pigmentation at 12/14 mg/kg/day (MF); Hepatocellular necrosis at 38/41 mg/kg/day (MF); Increased kidney weight at ≥30 mg/kg/day (F).	Harada, 1993b MRID 45014706

Study	Treatment-Related Histopathological Effects	Reference
Chronic Toxicity/ Oncogenicity Fischer F334 Rat 0, 3, 6, 24, 100 ppm (M); 0, 6, 60, 600 ppm (F); 0, 0.1, 0.2, 0.8, 3 mg/kg/day (M) 0, 0.2, 3, 25 mg/kg/day (F)	Not carcinogenic (MF); Increased liver weight and hepatocellular hypertrophy at 25 mg/kg/day (F); Increased kidney weight and renal tubular pigmentation at 3 mg/kg/day (M) and 25 mg/kg/day (F).	Harada, 1994a MRID 45000417
Oncogenicity CD-1 Mouse 0, 3, 10, 100 ppm 0, 0.3, 1.0, 10 mg/kg/day	Not carcinogenic (MF); **Hepatocellular hypertrophy with small eosinophilic granules at 10 mg/kg/day (MF) and microgranulomas at ≥ 1 mg/kg/day (F); Gastric mucosal hyperplasia at 10 mg/kg/day (M).**	Harada, 1994b MRID 45000418

Data obtained from Table 3 of MRID 46471103

3. *Not DNA reactive*

Five mutagenicity studies had negative results including an Ames assay, a gene mutation assay in mouse lymphoma L5178Y TK cells, an *in vivo* micronucleus assay in mouse bone marrow cells, an unscheduled DNA synthesis assay in rat hepatocytes, and an *in vitro* chromosomal aberration assay in Chinese hamster lung. Therefore, there is no concern for mutagenicity.

IV. Structure Activity Relationship (SAR)

Cyhalofop-butyl is in a class of pesticides (aryloxyphenoxy-propionate) that are known to be peroxisome proliferators. The primary tumor response for this class is the liver. Other chemicals in this class include clodinafop-propargyl, haloxyfop methyl, and diclofop methyl, all of which produced liver tumors. Clodinafop-propargyl had liver tumors with acceptable PPARα MOA data, but also had prostate tumors in rats and, therefore, its classification was "Suggestive" based on the prostate tumors. The other chemicals mentioned did not have any MOA data submitted and were classified based on the presence of liver tumors only. In addition, this class of pesticides does not show the triad tumor response (i.e. liver tumors, Leydig cell tumors, and pancreatic acinar cell tumors). While not in this class of chemicals, Fomesafen and Triclosan, which elicit peroxisome proliferation, were classified as "Not Likely to be Carcinogenic to Humans" based on the weight of evidence (WOE) that supported PPARα as the MOA for liver carcinogenesis in mice.

V. Classification of Carcinogenic Potential

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the cyhalofop-butyl is classified as "Not Likely to be Carcinogenic to Humans". This decision is based on the weight of evidence that supports activation of peroxisome proliferator activated receptor alpha (PPARα) as the primary mode of action in the liver. The data did not support a mutagenic mode of action. While the proposed mode of action is theoretically plausible in humans based on the availability of a functional PPAR-alpha receptor, hepatocarcinogenesis

via this mode of action is quantitatively implausible and unlikely to take place in humans based on quantitative species differences in PPARα activation and differences in toxicokinetics (Klaunig et al 2003). Cyhalofop-butyl is a weak peroxisome proliferator agonist in comparison to a potent liver tumor PPAR-alpha agonist such as WY14643.

VI. Quantification of Carcinogenic Potential

Quantification is not required.

VII. Overall Conclusion

The chronic toxicity/carcinogenicity study in rats (conducted at dose levels up to 10 mg/kg/day in males and females) and the carcinogenicity study in mice (conducted at dose levels up to 3.4 mg/kg/day in males and 25 mg/kg/day in females) showed no evidence of a tumorigenic response in either species. However, neither of these studies was considered to have reached a maximum tolerated dose. Mechanistic data in mice supported activation of PPARα as the primary mode of action in the liver for cyhalofop-butyl. Evidence of peroxisome proliferation was present at ≥5 mg/kg/day and significant liver toxicity (hepatocellular necrosis, increased liver weight, and moderate hypertrophy) was seen in the mechanistic studies at ≥50 after only 28 days of exposure. Dose levels ≥50 mg/kg/day would be considered excessive based on current EPA guidance. Morphological changes typical of a mitogenic MOA, including increased liver weight and liver hypertrophy, were seen in rat and mouse subchronic and chronic toxicity studies at doses of ≥25 mg/kg/day for rats and ≥10 mg/kg/day for mice. Therefore, given the fact that cyhalofop-butyl is not a liver toxicity/cancer concern for humans based on mechanistic information and the doses in the original chronic studies were approaching a maximum tolerated dose, repeating the chronic studies at higher doses would not provide useful information to the risk assessment and, therefore, is not being required. Accordingly, the quantification of cancer risk and the derivation of an RfD should not be based on liver effects since the PPARα rodent liver mode of action is not likely to occur in humans based on quantitative species differences in the differential regulation of genes involved in liver toxicity mediated by PPARα (e.g., Yang et al., 2007) and because cyhalofop-butyl is a weak rodent liver PPARα agonist.

VIII. References

MRID	CITATION
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